Bone, Tools, and Traditions
15th WBRG Meeting (Paris, 2024)
Organized by Nejma Goutas and Marianne Christensen
Paris, Société préhistorique française, 2026 (Séances de la Société préhistorique française, 24)
pp. 27–38
www.prehistoire.org
ISSN : 2263-3847 – ISBN : 978-2-9588382-2-5

Raw materials identifications: Developments and limits

Identification des matières premières : avancées et limites

Sonia O'Connor

Abstract: Confident identification of animal hard tissues used to produce archaeological objects is fundamental to their study. A detailed understanding of tissue formation and growth is essential to enable reliable identification by eye and by low-power microscopy. Bone and antler may be distinguished by the degree and form of their vascularisation and secondary remodelling, and each may retain something of their anatomical morphology. Ivories are non-vascular, and mostly characterised by the form of their incremental growth structures. Keratinous tissues may be distinguished by their varied layered or tubular microstructures, their original shape often having been remodelled by heat and pressure moulding. Analytical techniques based on elemental composition and vibrational spectrometry are largely non-destructive and assist in the characterisation of hard tissues. Analyses of DNA and peptide spectra require small-scale destructive sampling and can give precise species, but not skeletal element, identifications.
Keywords: Artefact, identification, visual, chemical, biomolecular, osseous, keratinous.

Résumé : Pour la plupart, les tissus durs des mammifères sont osseux ou kératiniques. Les matières dures d'origine animale, telles que les os, les bois de cervidé et l'ivoire, sont en grande partie constituées de protéines de collagène, rigidifiées par un minéral : la bioapatite. Les matières kératiniques, telles que la corne, le sabot, le fanon de baleine et l'écaille de tortue, sont en grande partie constituées de protéines kératiniques avec un faible composant minéral. Au sein de chaque groupe, les structures tissulaires sont distinctes, reflétant leurs fonctions biomécaniques et physiologiques du vivant de l'animal. L'intégration étroite de la bioapatite et du collagène dans les tissus osseux permet leur conservation dans divers environnements. Les tissus kératiniques sont, par contre, très sensibles à la biodétérioration et aux attaques chimiques. Lorsqu'ils sont en contact avec du métal (un manche de couteau par ex.), la corrosion de ce métal laisse des traces qui sont parfois préservées (minéraux préservés organiques – MPO). La kératine survit parfois dans des contextes d'enfouissement anaérobique gorgés d'eau ou dans des environnements desséchés ou gelés.
L'identification visuelle de ces matériaux dépend de la compréhension de l'histologie tissulaire, de l'apparence de la microstructure à l'échelle macroscopique et de la façon dont ces caractéristiques varient phylogénétiquement. Ce type d'identification n'implique ni un échantillonnage destructif, ni une préparation de la surface ou une altération chimique des objets. L'équipement requis est un microscope binoculaire de faible grossissement (x10-50), un éclairage de bonne qualité, une caméra avec fonction macro et/ou un microscope numérique.
L'objet est examiné en enregistrant sa taille, sa forme, la texture visuelle du matériau, s'il est fait d'une ou plusieurs pièces, ainsi que les traces présentes à sa surface, qu'il s'agisse de modifications anthropiques ou découlant de la biostratonomie et de la diagenèse. La première étape est d'identifier l'axe structurel principal du matériau : la plupart des matières dures d'origine animale sont anisotropes et présentent des caractéristiques différentes selon les plans. Des reliquats de formes anatomiques peuvent permettre l'identification précise de l'élément squelettique et même celle du taxon exploité. Toutes les caractéristiques diagnostiques relevant de l'identification d'un objet doivent être enregistrées et photographiées, car elles sont essentielles pour étayer les conclusions. Les guides d'identification mettent inévitablement l'accent sur les caractéristiques « typiques », mais, dans la pratique, certaines caractéristiques peuvent varier considérablement en fonction de facteurs agissant au niveau de l'individu ou de la population. Le façonnage, le polissage, la manipulation et l'usure des objets, l'application, intentionnelle ou non, d'huiles, de cires et de teintures, ou encore le fait de les chauffer ou de les brûler peuvent modifier leurs propriétés physiques, comme le processus de diagénèse au cours de l'enfouissement.

Les os et les bois de cervidé sont des tissus cellulaires hautement vascularisés, contrairement aux autres tissus rigides d'origine animale. Les variations du système vasculaire et de la matrice osseuse reflètent la fonction, la croissance et le remodelage de l'élément squelettique. Des restes de tissu spongieux ou de foramens nourriciers (pour ce qui est de l'os) peuvent permettre l'identification de l'élément anatomique. Le bois de cervidé combine un tissu compact dense enveloppant systématiquement un tissu spongieux, contrairement à toute autre forme de matière osseuse. L'identification des espèces est difficile pour les petits objets en bois de cervidé, mais les caractéristiques à la jonction du tissu compact et du tissu spongieux peuvent être informatives.
L'ivoire désigne la dentine prélevée dans n'importe quelle grande défense ou dent. Les ivoires sont acellulaires, non vasculaires et finement lamellaires. La croissance se fait par l'ajout progressif de minuscules couches de dentine à la surface de la cavité pulpaire, à partir desquelles des tubules dentinaires, de quelques microns de diamètre, irradient radialement et régulent le mouvement des fluides (principalement du plasma et des protéines de la pulpe dentaire) à travers la dent au cours de sa vie. Les ivoires sculptés peuvent être identifiables au niveau taxinomique, car la structure de la dentine reflète étroitement la forme de la cavité pulpaire et produit souvent des caractéristiques distinctives. Les caractéristiques des tubules dentinaires varient selon les ivoires et forment des macro-caractéristiques distinctives, telles que le motif Schreger de l'ivoire d'éléphant ou de mammouth.
Les tissus kératiniques sont acellulaires et morts. Ils peuvent être stratifiés, formés de tubules noyées dans une matrice, ou découler d'une combinaison de ces deux structures. La couleur de la kératine peut varier considérablement. Il s'agit d'un matériau thermoplastique naturel qui peut être complètement remodelé sous l'effet de la chaleur et de la pression. Comme pour les autres matières dures d'origine animale, les détails de la macro et de la microstructure, et leur effet sur la dégradation fournissent des informations permettant l'identification.
L'identification visuelle a ses limites, et d'autres techniques peuvent être utiles. Les méthodes d'analyse chimique spectroscopique (XRF) sont considérées comme non destructives et montrent la composition élémentaire, potentiellement fortement affectée par les changements diagénétiques. La spectroscopie vibrationnelle (FTIR, FT-Raman) donne des informations sur la composition et la structure moléculaire des matériaux organiques et inorganiques. Son application à l'étude des kératines est prometteuse. Les analyses biomoléculaires (ADN, protéomique) peuvent faciliter l'identification des espèces lorsqu'un échantillonnage destructif à petite échelle (10 à 50 mg) est acceptable. L'analyse de l'ADN permet une identification précise des espèces pour les matériaux cellulaires ; elle est utile pour les objets en os mais moins pour ceux en ivoire. La vitesse de détérioration de l'ADN peut être rapide, cela dépend de la température. La meilleure préservation se fait dans des conditions froides et stables, et pas nécessairement anaérobies. Les analyses protéomiques (ZooMS, SPIN) analysent les séquences peptidiques des protéines structurelles, qui sont plus persistantes dans les matériaux anciens, mais donnent souvent des identifications d'espèces moins précises.
Un défi commun à toutes les analyses biomoléculaires est de s'assurer que l'échantillon analysé est endogène à l'objet et non contaminé, et de garantir l'origine des échantillons de référence. Si les analyses biomoléculaires et spectroscopiques peuvent préciser l'identification visuelle de la matière, elles ne peuvent pas les remplacer et un examen visuel détaillé constitue toujours la première étape.
Mots-clés : artefact, identification, visuelle, biomoléculaire, osseuse, kératinique.

1. THE MATERIALS

Mammalian hard tissues fall into two groups: osseous and keratinous. Osseous materials, such as bone, antler and ivory, are largely collagen protein stiffened with bioapatite mineral, and are opaque to translucent off-white in colour. The term "bone" is sometimes used imprecisely to refer to any hard tissue of the musculo-skeletal system. In this paper, however, bone is used in its strict sense as a mineralised and vascular skeletal tissue, of which antler is a specialised form. Keratinous materials, such as horn, hoof, baleen and tortoiseshell, are largely keratin protein with minimal mineral component, flexible, often translucent and yellow-brown in colour, sometimes with additional melanin pigmentation. Within each group, the materials are chemically very similar but structurally distinct. This reflects the biomechanical and physiological functions of the tissue in life, its formation and growth.

The nano-scale, tight integration of bioapatite and collagen in osseous tissues allows their survival in diverse burial environments (Collins et al., 2002). In contrast, keratinous tissues are highly susceptible to biodeterioration and chemical attack, especially in alkaline conditions. Traces in metal corrosion (mineral preserved organics [MPOs]), attest to their widespread use, such as horn for knife and sword handles. Keratin may survive in anoxic waterlogged sites, particularly peat bogs, where acid pH and plant-derived biocides afford protection. Finds from desiccated environments, retreating glaciers and permafrost sites show how extensively keratinous tissues were utilised in antiquity.

2. VISUAL IDENTIFICATION

Visual identification requires understanding the histology of these tissues, how the microstructure is expressed on the macro-scale and how these features vary between families, genera and species. It is essentially an extension of looking critically at objects, observing and recording their detail. It does not involve destructive sampling, surface preparation or chemical alteration of objects, so the evidence for the identification remains intact and in-situ, and can be interrogated and reinterpreted without harm to the object. The equipment required is inexpensive and portable; good quality adapt-

able lighting, a camera with macro facility, a low-power binocular microscope and/or a suitable digital microscope (O'Connor, 2016).

The object is first examined, noting the overall size, shape and visual texture of the material, whether it is made from one or more pieces, and which surface features are natural, formed by working, or are the result of other taphonomic changes such as fracturing, gnawing or decay. Exploring the macrostructure of the material itself starts by establishing the long axis of the tissue, often detectable as a "grain", the direction of elongation of features such as blood vessels or striae. Being anisotropic, the appearance of the macrostructure of these materials depends on the angle of the surface. Confirming the orientation of an object to the long axis makes it easier to understand the macrostructure evidence and test possible identifications by predicting what features should appear on different surfaces.

The evidence from all these levels of examination is combined to build, test and refine the identification. Obviously, the survival of areas of gross morphology can enable the identification of a skeletal element and species, by comparison with zoological reference material. These may be quite subtle traces but still diagnostic. For example, pins may be made from modified pig fibulae but also cut from slivers of larger mammal longbone shafts to mimic the shape of pig fibulae. The two can be confidently separated as the medullary cavity of the fibula appears as a small central hole at the worked point of the pin or in transverse fractures of the shaft.

Visual identifications rarely rely on a single feature but integrate many pieces of evidence to make the difference between saying that an object is, for instance, ivory (dentine) or ivory cut from the tip of a lower medial incisor of a hippopotamus. In general, the more structural variation there is within the material of the object, the easier it is to refine the identifications. Small size can be an issue, unless a particularly characteristic feature has been encapsulated within the object. Like any analytical study, evidence supporting the identification should be recorded and reported to support the conclusions. Record images showing the object overall and indicating areas of interest, and macro images and/or photomicroscopy of diagnostic features facilitate recording and increase confidence in the interpretation of the evidence.

2.1. Identification criteria

The criteria used for visual identification of worked materials have been established over many decades mainly from the examination of polished transverse or longitudinal sections of contemporary specimens. Most identification guides are written for those working with historic objects or illegally trafficked wildlife. T. K. Penniman (1952), and B. Baker and colleagues (2020) discuss the identification of osseous materials, primarily ivories and their substitutes. M. C. Pedersen (2004) and M. Locke (2013) consider osseous and keratinous tissues, and other natural materials.

These guides inevitably emphasise "typical" features. In practice there can be considerable variation in the prominence, presence or absence of some features, driven by factors acting at the individual or population levels. Remodelling in response to disease or trauma can cause more confusion, as can the working and use of objects. Carving, polishing, handling and wear, the deliberate or accidental application of oils, waxes and stains, heating or burning can change physical properties such as strength, flexibility, surface texture, colour or translucency. However, some changes may also aid identification by enhancing the visibility of the micro- and macrostructure. Patterns of breakage, ageing and decay are particularly diagnostic as they are largely determined by the structure of the material.

The chemical and physical changes to these materials in archaeological contexts can be transformative to the point that little resemblance to contemporary or historical examples remains. Guides written specifically for identifying archaeological finds provide more pertinent information but differ in the materials they cover and in the detail of their descriptions depending on the preservation conditions, type and date of the sites where the authors gained their experience (O'Connor, 1987; Krzyszkowska, 1990; Deschler-Erb, 1998; Rijkelijkhuizen, 2008). This underlines the importance of building a comprehensive reference collection of examples and photographs of both contemporary and archaeological specimens, in very different states of preservation, whose identity has been verified.

2.2. Discrimination

2.2.1. Bone and antler

Bone and antler are highly cellular and metabolically active tissues infiltrated with blood vessels, unlike other animal hard tissues (fig. 1). The highly organised structure of compact mammal bone is similar across species but distinctly different to that of non-mammals. Variations in the detail of the vascular system and bone matrix reflect the function of the skeletal element and how it grows and is remodelled in life. Thus cetacean bone (fig. 1c), for instance, can be separated from terrestrial mammal bone (fig. 1a) and long bone material from that of flat-bones. Some estimate of the size and maturity of the animal can be gained from the dimensions of the object, the scale of the vascular system and features such as secondary osteons. Where remnants of spongy bone or features such as foramina have survived, the anatomical element may be identifiable but the species can remain elusive.

The combination of their temporary nature, spectacular growth, combination of dense bone cortex (or compact tissue) and spongy medulla (or cancellous tissue) and physical toughness sets antler apart from other bone. When working has removed the spongy medulla, antler can still be separated from bone by the macro-features of the cortex with its highly branched vascular system (fig. 1a and fig. 1b). Variations in the detail of the

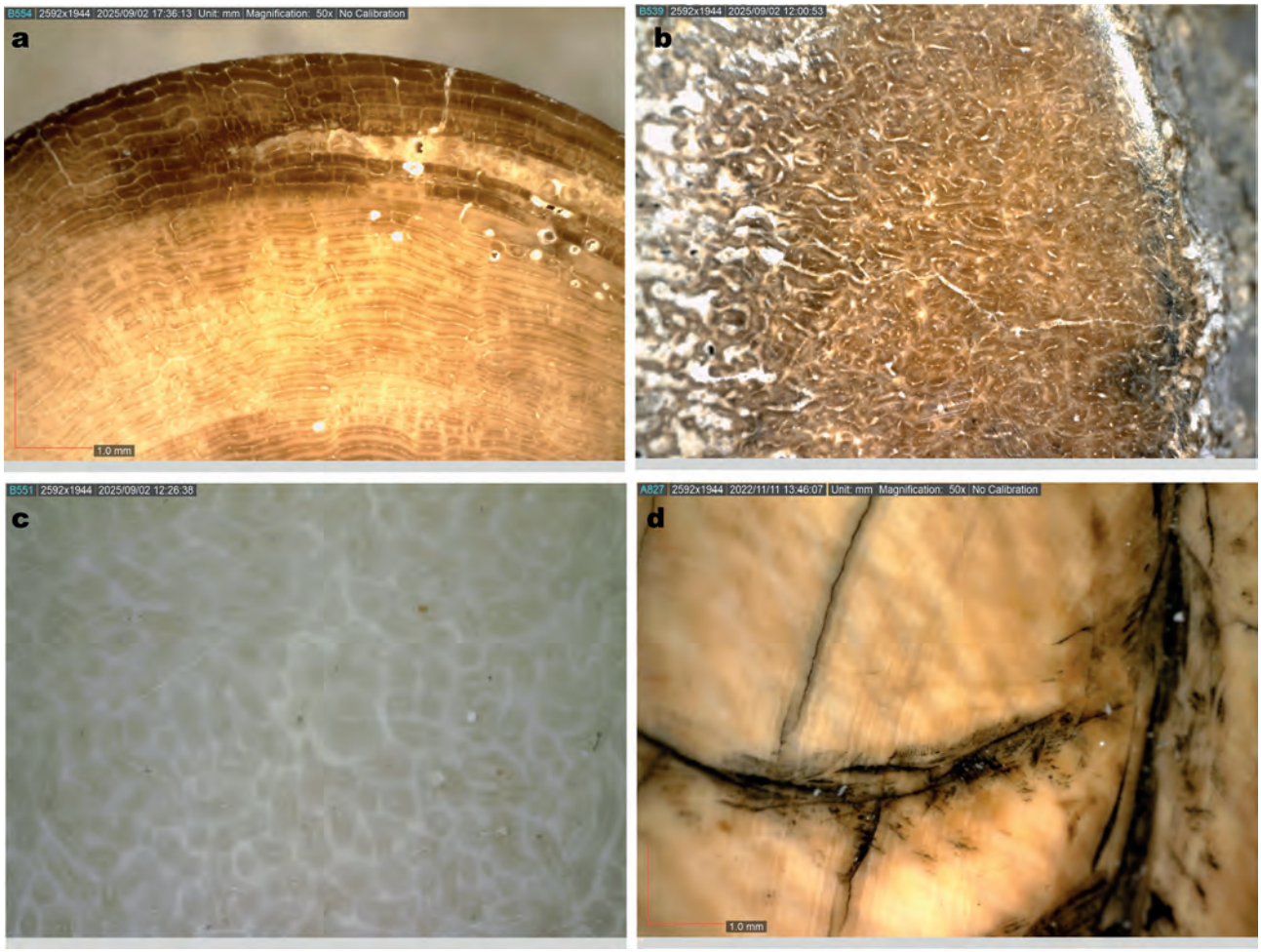

Fig. 1 – Photomicrographs of the transverse surfaces of vascular and non-vascular osseous tissues: a)The plexiform organisation of a red deer longbone shaft; b) The irregular, looped structure of red deer antler cortex; c) The primary and secondary osteon structure of bone from the rostrum of a mature sperm whale; d) The finely lamellar, non-vascular structure of dentine overlying the dark and light patches of the Schreger pattern of elephant ivory. The coarse, dark lines are tool-marks.

***Fig. 1** – Microphotographies des surfaces, en section transversale, des tissus osseux vasculaires et non vasculaires : a) organisation plexiforme d'un os long de cerf ; b) structure irrégulière et en boucle du cortex du bois de cerf ; c) structure primaire et secondaire des ostéons d'un os provenant du rostre d'un cachalot mature ; d) structure finement lamellaire et non vasculaire de la dentine recouvrant les zones sombres et claires du motif de Schreger de l'ivoire d'éléphant. Les lignes sombres et grossières sont des marques d'outils.*

medulla and cortex can indicate the use of tine, beam or material cut close to the burr, and even the season the antler was harvested. For example, red deer antler develops secondary infilling of the medulla in the region of the burr just before the antler is shed in the Winter. Species identification is difficult for objects without some evidence of the original size, morphology or surface detailing, but measurement of macro-structural features at the cortex/medulla boundary allows reliable separation of reindeer from red deer antler (Ashby, 2009; Lefebvre et al., 2016).

2.2.2. Ivories

Ivory is a term most commonly associated with elephant and mammoth tusks but can also refer to material cut from any large tooth, including those of hippopotamus, walrus, sperm whale, narwhal, killer whale and pig. Being dentine, ivories are acellular, non-vascular and finely lamellar (fig. 1d). Growth is by the addition of minute layers of dentine to the surface of the pulp cavity, and radially organised tubules regulate fluid movement through the tooth during life. These dentinal tubules are a few microns in diameter, very much smaller than blood vessels and only visible under very high magnification.

Despite these similarities, worked ivories are more readily identifiable to species than worked bone (fig. 2). The layering of dentine closely reflects the shape of the pulp cavity often producing distinctive features, such as the triangular organisation of the lamellae in hippopotamus lower canines in transverse sections (fig. 2c). If present in an object, the presence of enamel, details of the cementum/dentine junction, features formed along the central axis of the tooth above the pulp cavity and secondary structures formed within the pulp (fig. 2d) can all be diagnostic. The characteristics and path of the den-

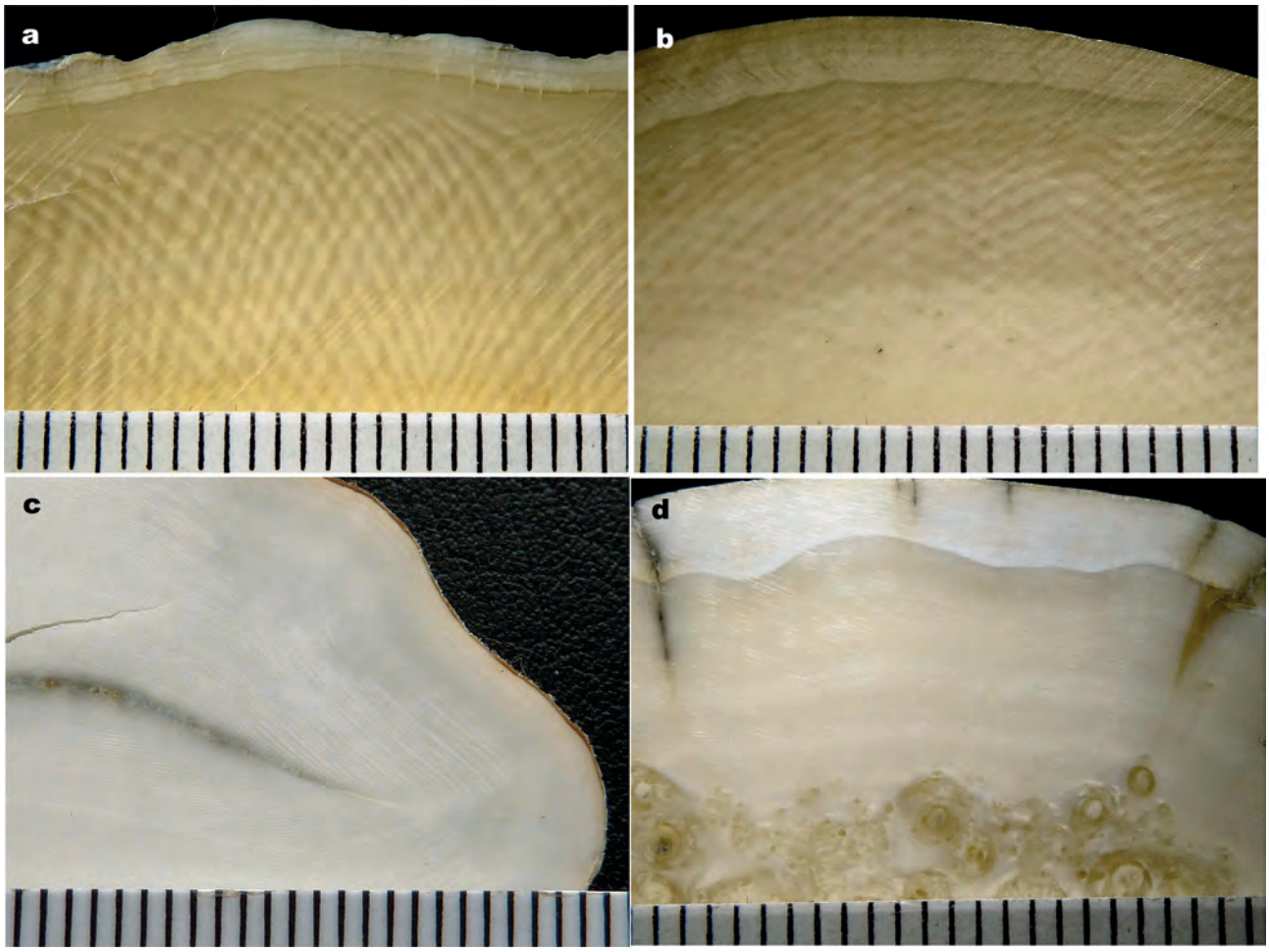

Fig. 2 – Tusk transverse sections of different species (mm scale): a-b) Mammoth (a) and elephant ivory (b; African), showing the marked differences in the outer angles of the Schreger pattern; c) The triangular organisation and the distribution of banding in hippopotamus canine ivory, d) The plain primary dentine and whorled secondary dentine of walrus ivory.

***Fig. 2** – Sections transversales de défenses de différentes espèces (échelle en mm) : a-b) ivoire de mammouth (a) et d'éléphant (b ; africain), illustrant les différences marquées dans les angles extérieurs du motif de Schreger ; c) organisation triangulaire et distribution des bandes dans l'ivoire d'une canine d'hippopotame ; d) dentine primaire simple et dentine secondaire verticillée de l'ivoire de morse.*

tinal tubules also vary in different ivories and although individual tubules are not visible to the eye, *en masse* they can produce visible macro-features such as patterns of concentric banding and variations in reflectance. The most widely recognised of these is the Schreger pattern of elephant and mammoth ivory (fig. 2a and fig. 2b). Measurement of the angles produced by this pattern at the periphery of a tusk transverse section have been used to determine extant and extinct proboscidean species (Trapani and Fisher, 2002; Baker et al., 2020). In practice this is rarely possible with worked objects because there are not enough suitable angles to measure, or none of the available surfaces is exactly transverse to the tusk, or there is no evidence conclusively placing the object at the outer edge of the tusk (fig. 1d).

2.2.3. Keratinous tissues

Keratinous tissues are tough, flexible, non-living tissues of the integument. In general, their form relates to their function and the shape of the bone or other tissue that supports the germinal layer but their detailed morphology can be species-specific, as illustrated by the huge variation in horn form across the ruminant artiodactyls. The process of cornification kills the germinal cells, which become compressed and stratified. In some tissues the mineral component can be concentrated in specific areas, affecting the opacity of the keratin but enhancing its stiffness and its resistance to wear. Keratinous tissues can be layered, like horn or marine turtle scutes (tortoiseshell), formed from tubules embedded in a matrix, like the walls of hooves and rhinoceros horn, or a combination of the two structures, as in baleen plates (fig. 3). Depending on the material and the species, the colour of translucent keratin varies considerably. Additional melanin pigmentation may be distributed evenly, within individual layers or in clusters, making patterns of blotching.

These distinguishing features may be visible on unworked tissue but are often obscured by weathering and are far more obvious in worked material. Polishing,

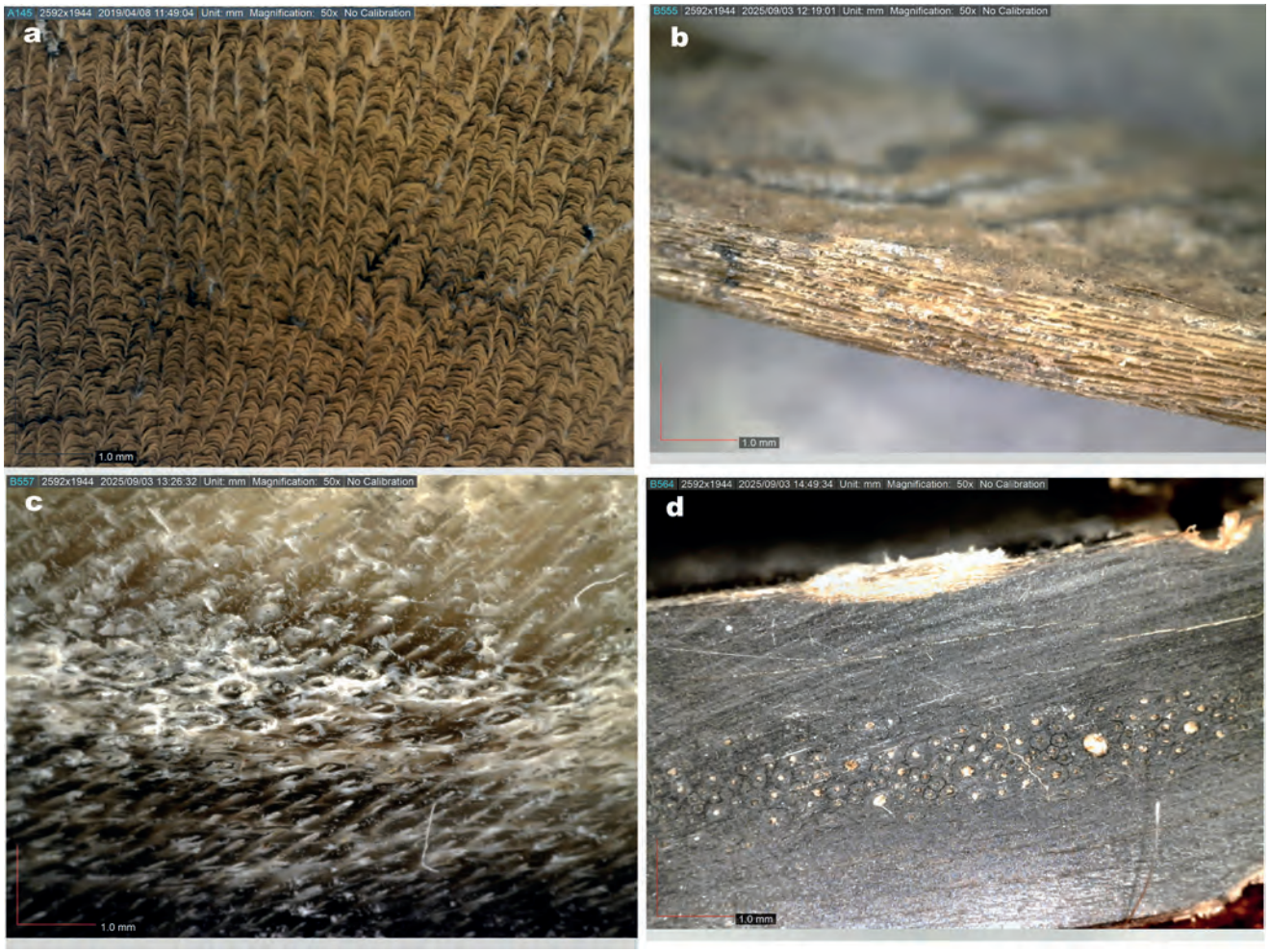

Fig. 3 – Photomicrographs of transverse sections through keratinous tissues. a) The strong radial structures reflect the concentrically organised, imbricated sheets of cattle horn. b) The flat, finely lamellar structure of tortoiseshell revealed by decay. c) The compressed tubular structure of the wall of a horse's hoof. d) The seam of thick-walled tubules and outer covering of lamellar keratin forming the tripartite structure of baleen (species unknown).

***Fig. 3** – Microphotographies de sections transversales à travers des tissus kératiniques : a) les structures radiales fortes reflètent des feuillets imbriqués et organisés de manière concentrique dans la corne de bovin ; b) la structure plate et finement lamellaire de l'écaille de tortue révélée par la décomposition ; c) la structure tubulaire comprimée de la paroi du sabot d'un cheval ; d) la couture de tubules à parois épaisses et de l'enveloppe extérieure de kératine lamellaire formant la structure tripartite du fanon (espèce inconnue).*

in particular, reveals the translucency and colour of keratin and patterns of pigmentation. However, working also loses the surface morphology and, because keratin is a natural thermoplastic, reshaping through heat and pressure can give objects shapes and dimensions alien to the original tissue.

Keratinous materials from archaeological contexts are identified in exactly the same way as osseous tissues. The pattern of growth, distribution of pigmentation, the underlying macroscopic features and how these affect the way the material deteriorates, together provide the evidence to distinguish the type of tissue, taxon and even species. Although translucency and natural colour are mostly lost to deterioration, the underlying structure can become more visible. Cattle horn, horn of sheep/goat, cattle or horse hoof, tortoiseshell and baleen, can still be distinguishable (O'Connor et al., 2015).

2.3. Limitations

Visual identification can distinguish different hard tissues, the skeletal element and taxon. However there are limitations, particularly to species identification, that are exacerbated by extensive working and the very small size of some objects. Because the structure of mammal bone varies little between species, visual identification often only allows us to say that, for instance, an object is worked from a strip of a rib, scapula, mandible or long bone shaft and that it comes from a small, medium or large sized mammal. The problems of species identification for keratinous tissues are similar, but in addition the histology of some materials has been less thoroughly researched. So, if species identification is essential to answer our research questions, how do we proceed?

3. OTHER APPROACHES TO IDENTIFICATION

3.1. Chemical analytical techniques

Several spectroscopic chemical analytical techniques that have established uses in archaeological science have been proposed for aiding the identification of animal tissues (table 1). Considered non-destructive, or at least minimally-invasive, these techniques use a beam of electromagnetic energy or ionised particles to interrogate the material. The beam is directed toward the object's surface, interacts with the target material, and changes to the beam are detected and analysed. These techniques vary in the range of elements detected, their sensitivity and the types of molecular structures they detect. The sample area may be from the nano- to the macro-scale, whilst scanning systems produce distribution maps of the substances detected. Some devices only provide qualitative or semi-quantitative results, further inhibiting comparisons across different studies.

Sometimes the choice of technique seems to be driven by the desire to find new applications for hard-earned equipment but there is also great appeal in a potential 'point and press' system, where the operator need have no specialist knowledge of the material or the analytical process. If an identification were immediately displayed, having presented the material to an analytical beam, this would have huge implications for the survey of large finds assemblages or for those working in the field of wildlife crime.

3.2. Discrimination

3.2.1. Osseous materials

Although osseous materials differ little in their major inorganic components, ratios of minor or trace components have been proposed as the basis for identification. The primary use has been to attempt to distinguish ivory from bone but species level identification has also been explored. Some have shown promise with natural history specimens or historical objects but perform less well with archaeological material and tend to be less discriminating than visual identification.

Magnesium/calcium ratio can be used to separate modern or historic specimens of bone and antler from most species of ivory, as the latter has significantly higher levels of magnesium. As a result of diagenetic changes, the magnesium content reduces overtime. The rate of change is much faster for dentine than bone in the same burial environment, and eventually they become indistinguishable (Müller and Reiche, 2011). The separation of archaeological antler or terrestrial bone from marine bone using strontium/calcium ratios plotted against fluorine content, proved successful for the two palaeolithic cave sites reported by K. Müller and I. Reiche (2011). However, if diagenetic changes led to the differential loss or take-up of either element, the technique would be compromised.

Diagenetic changes would seem to be behind the misidentification of one of the 11 Lewis Chessmen held at the National Museum of Scotland (NMS). Found on the Isle of Lewis, the 94 piece hoard of 13th C gaming pieces were mostly carved in walrus ivory though sperm whale ivory was also used. Qualitative differences in the calcium and strontium values of the NMS chessmen were used to assign species identifications (Tate et al., 2011). For 10 of the chessmen this was consistent with the previous visual assessments—nine as walrus ivory and one as sperm whale ivory. However, the knight (N27) had the lowest calcium ratio of all, indicating whale ivory despite having the shape and solidity of a section of walrus tusk. Clearly the authors wrestled with the contradictory evidence but concluded that it was whale ivory because the "pattern of the base [was] less diagnostic" than the other walrus ivory pieces and the "chalk-like" appearance of its degraded surface resembled that of the other whale ivory chessman (Tate et al., 2011, p. 252). However, the photograph (their figure 3b, p. 253) of the base of N27 appeared to show a pulp cavity filled with whorled secondary dentine characteristic of walrus ivory. After examining these chessmen first-hand, this author has been able to confirm that N27 is walrus ivory. The macro structure is unambiguous although a little obscured by surface degradation. In their experimental work, J. Tate and colleagues (2011) demonstrated that fungi could leach calcium from dentine, forming similar surface damage to that seen on the chessmen, but this did not lead them to question their use of Ca/Sr ratios as a means of discriminating ivory materials.

Technique	Dectecting
XRF (X-ray fluorescence)	Elemental analysis of inorganic materials
XRD (X-ray diffraction)	Elemental analysis and crystalline molecular structures
PIXE (particle-induced X-ray emission or proton-induced X-ray emission)	Chemical composition, molecular structure and material thickness
FT-IR (Fourier Transform infra-red spectroscopy)	Chemical composition and structure, including complex organic molecules

Table 1 – Chemical analytical techniques for animal hard tissue identification.
***Tabl. 1** – Techniques d'analyse chimique pour l'identification des tissus durs d'origine animale.*

Vibrational spectroscopy, such as FTIR (Fourier Transform InfraRed spectroscopy) and FT-Raman Spectroscopy, produces complex spectra that provide information on the composition and molecular structure of both organic and inorganic components of osseous and keratinous tissues. Most FTIR techniques require some surface preparation or removal of a small sample (e.g. 10 mg), depending of the equipment used, whilst FT-Raman Spectroscopy does not. Both techniques have shown potential in characterising contemporary and historical osseous tissues (Brody et al., 2001; Turner-Walker and Xu, 2014; Shepherd et al., 2024). Variations within and between species produce overlaps that can only be resolved statistically.

3.2.2. Keratinous tissues

The keratinous tissues are less studied and less well resolved, although the division between the helical form of the alpha-keratin of mammals and the pleated beta-keratins of amphibians and birds is clear. Using DRIFTS (Diffuse Reflectance Infrared Fourier Transform Spectroscopy) and discriminant analysis, E. O. Espinoza and colleagues (2007) successfully separated bovid horn and tortoiseshell samples. G. Turner-Walker and B. Y. Xu (2014), notes that different areas of the same keratinous tissue gave different FTIR responses and that working can produce profound change to material crystallinity, probably indicating the use of heat and pressure in their processing. H. G. M. Edwards et al. (1998) produced FT-Raman spectra of keratinous tissues that again highlighted the differences between tortoiseshell and mammalian keratin but the differences between the samples of mammalian tissues were extremely subtle.

3.3. Limitations

Testing vibrational spectroscopy on archaeological material, almost exclusively limited to osseous tissues, has not been promising. FTIR absorption bands produced for archaeological antler and ivory plot well outside the areas of variation of modern specimens (Turner-Walker and Xu, 2014). Attempts to provide species identification of ivories with FT-Raman spectroscopy have highlighted the very different preservation of the chemistry in different burial environments (Edwards et al., 2006; Edwards and O'Connor, 2012). Mammoth ivory from permafrost sites can provided spectra comparable to that from modern elephant but ivory surviving in other burial conditions may produce spectra that do not even resemble osseous tissues. Decay can lead to the absence of the organic components whilst contamination from the burial environment produces noisy spectra with levels of background emission high enough to compromise recognition of the bioapatite (O'Connor et al., 2011).

The literature in this area is pervaded by examples of "bad science" including the analysis of single specimens to represent an entire species, the use of unprovenanced material, lack of proper controls or validation of identifications by any other means. The chemical differences between samples or groups of samples are often small and if we do not know what is driving these differences we do not know what they mean. Beyond species differences, factors such as state of health, age, sex, lactation and diet, may all affect the attributes of the mineral and organic components. Analysis of greater numbers of better documented specimens may improve our understanding but it may also eliminate some of the apparent differences between different species in the same tissue. As to archaeological material, diagenetic changes can be so transformative that it seems unlikely that these approaches alone could ever provide reliable species identification.

4. BIOMOLECULAR ANALYSIS

Where species identification is crucial, biomolecular analysis is the only viable approach, either through DNA analysis or proteomics.

Depending on the quantity and quality of ancient DNA, it is possible to identify species, sex, population traits, familial information and even individual characteristics. Questions need to be well defined. DNA analysis is expensive and potentially destructive, usually requiring samples around 500 mg for archaeological material though new techniques hold out the possibility of much smaller samples (50 mg: Tejero et al., 2024). The sample is drilled from the interior of the object to limit surface destruction, reduce problems of DNA contamination and seeking better preserved DNA than is found at the surface (Hollund et al., 2017).

Bone is rich in cells and therefore DNA, but dentine is acellular so DNA analysis is not a useful tool for identifying ivories unless the bone-like, cellular cementum outer covering is present on the object. Even when archaeological material appears well preserved, there is no guarantee that the preservation of the DNA will be sufficient to provide even taxon information. The rate of DNA deterioration is temperature dependant and humid, warm archaeological deposits are particularly damaging. The best DNA preservation is generally found in cold, stable conditions such as permafrost or deep caves. For most situations, proteomic analysis may be a better option for taxon identification.

Proteomic analysis is used to sequence the peptides of the tissue's structural protein, which can persist when DNA does not. ZooMS, Zooarchaeology by Mass Spectrometry, originally developed for the identification of bone (Buckley et al., 2009), has become the most common procedure for the peptide mass fingerprinting (PMF) of historical and archaeological materials containing collagen. Although collagen is highly conserved in evolutionary terms, some portions show more peptide sequence variability than others and these are targeted by ZooMS. The collagen is extracted and broken down enzymatically to produce a spectrum showing the intensity of peptides of different molecular mass. This is the "fingerprint" of

the collagen and taxon identification is gained by comparing this spectrum with those gained from known species. As wet extraction of the collagen was seen as potentially damaging to historic and archaeological objects, a dry, non-invasive sampling technique was developed using a polyester eraser (Fiddyment et al., 2015). Typically requiring a 5 mg sample, the negligible impact of this technique proved to be acceptable even to archivists when sampling historic parchment documents. More recently, more efficient sampling using microabrasive film (Kirby et al., 2020) in combination with improvements in extraction and sequencing and the growth of the reference libraries of known protein sequences mean that animals can increasingly be identified to species level.

One obvious hazard with such very small samples is to be certain that the sampled material is endogenous to the object, and not surface contamination. On one occasion a clearly caprine radius was misidentified by ZooMS as bear (T. O'Connor *pers. comm*.) and three beads from the prehistoric site of Skara Brae, Orkney, UK, that ZooMS identified as "cattle" were clearly a section of deer metatarsal, a piece of cetacean bone and a female pig canine. In these cases, there was sufficient evidence to make a confident visual identification. Real problems arise when bone is sufficiently fragmented or modified to have lost all indicators of skeletal element and taxon—the most likely reason for undertaking ZooMS in the first place. K. McGrath and colleagues (2019) and A. Monticone and colleagues (2024) demonstrated that enough powder can be generated by archaeological material to enable ZooMS identifications to be made by sampling the internal surfaces of storage bags, highlighting the potential for the contamination of objects in reused packaging.

ZooMS has become a very powerful tool for species identification. Although it is still not possible to distinguish between red deer, fallow deer and elk, it is possible to identify most of the main sources of worked bone material to species, to separate pinnipeds from cetaceans and many cetaceans to species. A new study has apparently had success distinguishing contemporary African and Asian elephant ivory, although this has yet to be published or demonstrated with archaeological material. A new proteomic technique, SPIN (Species by Proteome Investigation) promises enhanced discrimination (Rüther et al., 2022) but may not always perform better than ZooMS on poorly preserved archaeological material (Hansen et al., 2024). The downside is that the sampling is more invasive. 50 mg is ten times that needed for ZooMS, though still appreciably less than often required for DNA.

PMF techniques (Peptide Mass Fingerprinting) are also capable of providing taxon identification of keratinous tissues (Solazzo et al., 2013). Despite the often poor condition of archaeological material and the limited availability of keratin sequences in public databases for comparison, identification to genus and even species can still be achieved (O'Connor et al., 2015). The sample size is of the order of 10 mg and processing of keratin samples is similar to that of collagen for ZooMS but with additional steps.

CONCLUSIONS

Analytical techniques are steadily enhancing our ability to put species names to objects. However, visual identification is still the only approach that can provide skeletal element information and, depending on the archaeological questions, understanding which parts of which species were chosen as artefact raw materials may be important. Proteomics may indicate elephant but cannot distinguish ivory from bone and an identification as deer could be bone or antler. It is the same for keratinous tissues; cow hoof, for instance, would be indistinguishable from cow horn. Even when visual examination cannot confirm species, it can help to frame research questions and the best way to answer them, indicating which analytical technique might be the most appropriate and identifying the most suitable sampling site. Taken together, visual identification followed by biomolecular analysis provides the most effective combination currently available for the identification of animal hard tissues worked into artefacts.

Acknowledgements: My thanks go to M. Christensen, N. Goutas and the ICAZ Worked Bone Research Group for the invitation to give this paper in Paris. I would also like to thank L. Benoit for her help with the abstract and K. McGrath, A. Coutu and M. Rosser-Owen for bringing me up to speed with the latest developments in proteomic sampling and sequencing. Finally, my thanks go to T. O'Connor for his help in the preparation of this paper.

Sonia O'Connor
University of Bradford
Bradford, UK
s.oconnor@bradford.ac.uk
soniasinbox@gmail.com

REFERENCES

ASHBY S. P. (2009) – Combs, contact and chronology: reconsidering hair combs in early-historic and Viking-Age Atlantic Scotland, *Medieval Archaeology*, 53*,* 1, pp. 1–33.

BAKER B., JACOBS R., MANN M., ESPINOZA E., GREIN G. (2020) – *CITES Identification Guide for Ivory and Ivory Substitutes*, Washington DC, World Wildlife Fund inc (4th edition), 97 p.

BRODY R. H., EDWARDS H. G., POLLARD A. M. (2001) – Chemometric methods applied to the differentiation of Fourier-transform Raman spectra of ivories, *Analytica Chimica Acta*, 427, 2, pp. 223–232.

BUCKLEY M., COLLINS M., THOMAS-OATES J., WILSON J. C. (2009) – Species identification by analysis of bone collagen using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry, *Rapid Communications in Mass Spectrometry*, 23, 23, pp. 3843-3854.

COLLINS M. J., NIELSEN-MARSH C. M., HILLER J., SMITH C. I., ROBERTS J. P., PRIGODICH R.V., WESS T. J., CSAPO J., MILLARD A. R., TURNER-WALKER G. (2002) – The survival of organic matter in bone: a review, *Archaeometry*, 44*,* 3, pp. 383–394.

DESCHLER-ERB S. (1998) *Römische Beinartefakte aus Augusta Raurica. Rohmaterial, Technogie, Typologie und Chronologie*, Augst, Römerstadt Augusta Raurica, 423 p.

EDWARDS H. G. M., O'CONNOR S. (2012) – Archaeological ivories: A challenge for analytical Raman spectroscopy, Chapter 16 in H. Edwards and P. Vandenabeele (eds.), *Analytical Archaeometry*, London, The Royal Society of Chemistry, pp. 449–467.

EDWARDS H. G. M., HUNT D. E., SIBLEY M. G. (1998) – FT-Raman spectroscopic study of keratotic materials: Horn, hoof and tortoiseshell, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 54*,* 5, pp. 745–757.

EDWARDS H. G. M., BRODY R. H., HASSAN N. F. N., FARWELL D. W., O'CONNOR S. (2006) – Identification of archaeological ivories using FT-Raman spectroscopy, *Analytica Chimica Acta*, 559, 1, pp. 64–72.

ESPINOZA E. O., BAKER B. W., BERRY C. A. (2007) – The analysis of sea turtle and bovid keratin artefacts using drift spectroscopy and discriminant analysis, *Archaeometry*, 49, 4, pp. 685–698.

FIDDYMENT S., HOLSINGER B., RUZZIER C., DEVINE A., BINOIS A., ALBARELLA U., FISCHER R., NICHOLS E., CURTIS A., CHEESE E., TEASDALE M. D. (2015) – Animal origin of 13th century uterine vellum revealed using noninvasive peptide fingerprinting, *Proceedings of the National Academy of Sciences*, 112*,* 49, pp. 15066-15071.

HANSEN J., DEKKER J., TROCHÉ G., FAGERNÄS Z., OLSEN J. V., SEGUI M. S., WELKER F. (2024) – A comparative study of commercially available, minimally invasive, sampling methods on Early Neolithic humeri analysed via palaeoproteomics, *Journal of Archaeological Science*, 167, 106002.

HOLLUND H. I., TEASDALE M. D., MATTIANGELI V., SVERRISDÓTTIR O. Ó., BRADLEY D. G., O'CONNOR T. (2017) – Pick the right pocket. Sub-sampling of bone sections to investigate diagenesis and DNA preservation, *International Journal of Osteoarchaeology*, 27*,* 3, pp. 365–374.

KIRBY D. P., MANICK A., NEWMAN R. (2020) – Minimally invasive sampling of surface coatings for protein identification by peptide mass fingerprinting: A case study with photographs, *Journal of the American Institute for Conservation*, 59, 3-4, pp. 235–245.

KRZYSZKOWSKA O. (1990) – *Ivory and related materials*, London, Institute of Classical Studies, 109 p.

LEFEBVRE A., ROCHEFORT G. Y., SANTOS F., LE DENMAT D., SALMON B., PÉTILLON J. M. (2016) – A non-destructive method for distinguishing reindeer antler (*Rangifer tarandus*) from red deer antler (*Cervus elaphus*) using X-ray micro-tomography coupled with SVM classifiers, *PLoS ONE*, 11, 2, e0149658.

LOCKE M. (2013) – *Bone, Ivory, and Horn Identifying Natural Materials*, Atglen PA, Schiffer Publishing Limited, 280 p.

MCGRATH K., ROWSELL K., GATES ST-PIERRE C., TEDDER A., FOODY G., ROBERTS C., SPELLER C., COLLINS M. (2019) – Identifying archaeological bone via non-destructive ZooMS and the materiality of symbolic expression: Examples from Iroquoian bone points, *Scientific reports*, 9, 1, e11027.

MONTICONE A., PANERO E., HERITIER E., PERGOLIZZI B., BELLO F. D., MECARELLI E., BOANO R., DE VINGO P., CODLIN M., PESSIONE E., DEMARCHI B. (2024) – Combing through museum collections. A "museomic" application of ZooMS, *International Journal of Osteoarchaeology*, 34, 2, p. e3295.

MÜLLER K., REICHE I. (2011) – Differentiation of archaeological ivory and bone materials by micro-PIXE/PIGE with emphasis on two Upper Palaeolithic key sites: Abri Pataud and Isturitz, France, *Journal of Archaeological Science*, 38, 12, pp. 3234–3243.

O'CONNOR S. (1987) – The identification of osseous and keratinaceous materials at York, in K. Starling and D. Watkinson (eds.), *Archaeological bone, antler and ivory*, London, United Kingdom Institute for Conservation, pp. 9–21.

O'CONNOR S. (2016) – The COWISHT project enhancing the identification of artefact raw materials, *Cuadernos del Instituto Nacional de Antropologia y Pensamiento Latinoamericano – Series Especiales*, 3*,* 2, pp. 4–22.

O'CONNOR S., EDWARDS H. G., ALI E. (2011) – An interim investigation of the potential of vibrational spectroscopy for the dating of cultural objects in ivory, *ArcheoSciences. Revue d'archéométrie*, 35, pp. 159–165.

O'CONNOR S., SOLAZZO C., COLLINS M. (2015) – Advances in identifying archaeological traces of horn and other keratinous hard tissues, *Studies in Conservation*, 60, 6, pp. 393–417.

PEDERSEN M. C. (2004) – *Gem and ornamental materials of organic origin,* Oxford, Elsevier Butterworth-Heinemann, 268 p.

PENNIMAN T. K. (1952) – *Pictures of ivory and other animal teeth, bone and antler, with a brief commentary on their use in identification*, Oxford, University of Oxford (Pitt Rivers Museum Occasional Papers on Technology, 5), 40 p.

RIJKELIJKHUIZEN M. (2008) – *Handleiding voor de determinatie van harde dierlijke materialen: Bot, gewei, ivoor, hoorn, schildpad, balein en hoef*, Amsterdam, Amsterdam University Press, 112 p.

RÜTHER P. L., HUSIC I. M., BANGSGAARD P., GREGERSEN K. M., PANTMANN P., CARVALHO M., GODINHO R. M., FRIEDL L., CASCALHEIRA J., TAUROZZI A. J., JØRKOV M. L. S. (2022) – SPIN enables high throughput species identification of archaeological bone by proteomics, *Nature communications*, 13, 1, 2458.

SHEPHERD R. F., LISTER A. M., ROBERTS A. M., TAYLOR A. M., KERNS J. G. (2024) – Discrimination of ivory from extant and extinct elephant species using Raman spectroscopy: A potential non-destructive technique for combating illegal wildlife trade, *PLoS ONE*, 19, 4, e0299689.

SOLAZZO C., WADSLEY M., DYER J. M., CLERENS S., COLLINS M. J., PLOWMAN J. (2013) – Characterisation of novel α-keratin peptide markers for species identification in keratinous tissues using mass spectrometry, *Rapid Communications in Mass Spectrometry*, 27, 23, pp. 2685-2698.

TATE J., REICHE I., PINZARI F., CLARK J., CALDWELL D. (2011) – History and surface condition of the Lewis Chessmen in the collection of the National Museums Scotland (Hebrides, late 12th-early 13th centuries), *ArcheoSciences*, 35, pp. 249–258.

TEJERO J. M., CHERONET O., GELABERT P., ZAGORC B., ÁLVAREZ-FERNÁNDEZ E., ARIAS P., AVERBOUH A., BAR-OZ G., BARZILAI O., BELFER-COHEN A., BOSCH M. D. (2024) – Cervidae antlers exploited to manufacture prehistoric tools and hunting implements as a reliable source of ancient DNA, *Heliyon*, 10, 11, e0309789.

TRAPANI J., FISHER D. C. (2003) – Discriminating proboscidean taxa using features of the Schreger pattern in tusk dentin, *Journal of archaeological science*, 30, 4, pp. 429–438.

TURNER-WALKER G., XU B.-Y. (2014) – Identification of animal hard tissues using Fourier transform infrared spectroscopy, in J. Bridgland (ed), *Natural History collections. Preprints ICOM-CC 17th Triennial Conference,* Paris, International Council of Museums, pp. 1–11 [posted on Research Gate].